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Spun Down 1.2l cells - GS-3 - 7000 RPM - 30 min - Decant
- Dissolve 86 grams cells in 25 ml Crude BFR → (Pg 7)

Sonicate 4 X 3.5 30 sec. (+) 6 at 4.5 30 sec each

A 540 1:200 dil'n

Crude - .98 ≅ 70% crude → Heat 15 min @ 88°C.
Finale - .13 cool 10 min / ice

ADD 0.4% PEI (1.2 mls 10% stock) - STIR 15 min
Spun in SS-34 9K - 30 min - Decant sup.
7.55gms / 20 mls → ADD NH_4SO_4 - 308 g/l (48% cut) - STIR 4°C 45 min
- Spun down in SS 34 1/2 hr 12K - Decant sup, save for activity.
Resuspend pellet in 8 mls BFR. A - Dialyze o/n 4°C. vs. 500 mls

BFR. A
25mM Tris-7.4
8% glycerol
0.5mM EDTA
10mM KCl
5mM BME (1.3 mls)

BFR B (High salt gradient)
Same (+) 2M KCl.
TOSO-650 Heparin.
Pour 5 ml column Equilibrate w/ A.
Load at 0.5 ml/min
wash w/ 8 VTs - or till baseline at 1.5 ml/min

*

Change dialysis buffer once -

Bump 4 ml TOSO 650 Heparin - 4M Gu HCl - 3M NaCl 10 vts
Wash with H_2O -
Equilibrate w/ BFR A - Load = (1.4 mls) Load = 7.1 mls -
Load - .75 ml/min.
[illegible]

10 VT gradient A→B - 8 ml fractions

Mix - premade by HG. - Stored @ -20°C - same rxn mix as for native

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Witnessed & Understood by me,	Date	Invented by	Date
[signature]	2/27/95	[signature]	12-5-94
		Recorded by	